Bruchez et al.
U.S.S.N. 10/735,608

Docket. No.: QDC 0014.20

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: Bruchez *et al.*

Title: METHOD FOR ENHANCING TRANSPORT OF SEMICONDUCTOR NANOCRYSTALS ACROSS BIOLOGICAL MEMBRANES

Appl. No.: 10/735,608

Filing Date: 12/12/2003

Examiner: Pensee T. Do

Art Unit: 1641

DECLARATION OF JOSEPH TREADWAY UNDER 37 C.F.R. § 1.132

I, Joseph Treadway, state and declare that:

1. I received my Ph.D. in Chemistry from the University of North Carolina at Chapel Hill, in 1998, and focused my studies on Inorganic Chemistry.

2. I am employed by Invitrogen Corporation and I am currently the Technical Area Manager for Nanosciences. I was formerly employed by Quantum Dot Corporation from July, 1999 to October 2005 where I was a Principal Scientist. I am very familiar with semiconductor nanocrystals, including the transport of semiconductor nanocrystals across biological membranes, having worked in this discipline for seven years. I have coauthored numerous patents and publications in the field of semiconductor nanocrystals, including US Patent Application Numbers: 10/985,249; 11/011,827; 10/726,716; 10/807,616; 10/198,635 (US Patent No. 6,815,064); 09/827,013 (US Patent No. 6,734,420); 10/032,809 (US Patent No. 6,682,596), 10/263,366; and 09/751,670.

3. I have reviewed and understand experiments on the transport of cationic polymer-bound nanocrystals across biological membranes, including Examples 1-16 described in U.S. Patent Application No. 10/735,608 ("the '608 Application").

4. I have reviewed and understand U.S. Patent No. 6,306,993 (Rothbard *et al.*), US Patent No. 5,652,122 (Frankel *et al.*) and US Patent No. 6,306,610 (Bawendi *et al.*). In my opinion, Rothbard *et al.* describes the conjugation of drugs such as paclitaxel (Example 9) and proteins such as ovalalbumin (Example 12) to polymers containing multiple arginine subunits. Rothbard *et al.* does not teach how to functionalize any and all macromolecules for transport across membranes. Frankel *et al.* describes the transport of polypeptides, nucleic acids, and polysaccharides using HIV tat polypeptides. Like Rothbard *et al.*, Frankel *et al.* does not teach how to functionalize any and all macromolecules for transport across membranes. Bawendi *et al.* describes various

semiconductor nanocrystals capable of binding multiple target agents such as antigens or antibodies. Bawendi *et al.* does not describe how to transport nanocrystals across biological membranes.

5. Semiconductor nanocrystals, such as those described in the '608 Application, encounter unique solubility, reactivity, and transportability issues not associated with proteins or drugs. Notably, nanocrystals are very rigid as compared with proteins such as ovalalbumin. The nanocrystals have a very high ionic density and a hydrophobic core, which makes dissolution in biological solutions very difficult. The nanocrystals generally have an inflexible diameter of approximately 2-40 nm. The nanocrystals are also comprised of semiconductor materials, such as cadmium selenide and zinc sulfide, which are not generally present in biological systems and certainly not present in proteins or small molecules. These unique properties, particularly insolubility, inflexibility, and size make the transport of nanocrystals across biological membranes exceptionally difficult.

6. Prior to experimentation, it is very difficult to predict how a substituent will affect the electronic properties of a nanocrystal, *per se*. Moreover, the dissolution and subsequent transport of nanocrystals across a biological membrane, in a solution necessary to maintain a biological membrane, makes suitable substituent predictions even more difficult. In particular, properties such as solubility, high rigidity, core hydrophobicity, large particle diameter and ionic density adversely affect the nanocrystal's ability to associate with substituent groups and biological membranes. For example, the large diameter and ionic density correlate to a higher quantity of sites for polymer association as compared with proteins or small molecules. The net charge of the nanocrystal and ability to interact with negative charges on the surface of a biological membrane is thereby affected. Also, the rigidity of the particles translates to a much less pliant surface for accommodation of the polymers and subsequent transport across the biological membrane, as compared with proteins such as ovalalbumin. Particularly, proteins are able to constrict and bend in sections in order to wriggle through a membrane, whereas nanocrystals cannot. The hydrophobic portion of the nanocrystals is also capable of adhering to non-polar surfaces, such as membranes, through hydrophobic interactions. Finally, surface properties and the potential for fluorescencent quenching caused by interactions of the cationic polymer and nanocrystal coating must also be considered. These issues are not encountered by proteins, nucleic acids and small molecule drugs. Accordingly, prior to experimentation or review of the '608 Application, I could not have predicted that cationic polymers bound to nanocrystals would result in a complex with a sufficient ability to cross biological membranes.

7. In my opinion, the coexistence of Bawendi *et al.* and Rothbard *et al.* or Frankel *et al.* fails to provide any plausible suggestion that cationic polymers would improve the ability of nanocrystals to cross biological membranes. Furthermore, neither Bawendi *et al.*, Rothbard *et al.*, nor Frankel *et al.* (nor a combination thereof) describe how such molecules could be made.

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8. I hereby acknowledge that willful false statements and the like are punishable by fine or imprisonment, or both (18 U.S.C. § 1001) and may jeopardize the validity of the above-referenced application or any patent issuing thereon. All statements made of declarant's own knowledge are true and all statements made on information and belief are believed to be true.

Joseph Treadway, Ph.D.

June 14, 2006
Date